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Full Length Article

Characterization of an Emerging Isolate of Watermelon Mosaic Virus in Turkey

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Abstract

A watermelon mosaic virus isolate (WMV-Tr) was obtained from a naturally infected watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) plant with mosaic, mottle and leaf deformation symptoms collected in the major cucurbit-growing area in Adana province of Turkey during a survey conducted in May 2009. DAS-ELISA and RT-PCR showed the presence of watermelon mosaic virus (WMV, *Potyvirus*) in the sample. WMV-Tr was characterized biologically and its partial coat protein genome sequence was established. WMV-Tr had biological properties similar to those reported for the WMV isolates from different parts of the world. WMV-Tr belonged to molecular group 3, containing Asian isolates of WMV as well as isolates currently emerging in different parts of the world including Europe. This suggests recent emergence of Group 3 isolates in Turkey. © 2015 Friends Science Publishers

Keywords: Watermelon; WMV; ELISA; RT-PCR; Emergence; Phylogenetic analysis

Introduction

Fruit bearing vegetables are one of the most economically important crops grown in Adana province in Turkey. Acording to reports of Turkish Statistical Institute in 2009, the estimated annual production of vegetable crops in Adana is approximately 1,42 million tons covering approximately an area of 32,900 ha. *Cucurbitaceae* family constitutes about 70% of the crops grown. Watermelon ranks first (841,805 tons), followed by melon (103,765 tons), cucumber (20,890 tons) and squash (15,563 tons) in Adana province (Anonymous, 2009).

Many factors decrease quantity and quality of the production in cucurbits as well as in other crops. Virus diseases are one of the main factor limiting cucurbit production. At least 60 viruses can infect plants in the *Cucurbitaceae* family, and new virus species on these hosts are described every year (Provvidenti, 1996; Lecoq and Desbiez, 2012; Romay *et al*., 2014). Cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), papaya ringspot virus (PRSV), and watermelon mosaic virus (WMV) are the most frequent and economically important viruses on a worldwide basis (Lecoq and Desbiez 2012).

Watermelon mosaic virus (WMV) belongs to the genus *Potyvirus* in the family *Potyviridae*. It has flexuous filamentous particles. The virus can cause economically important losses in thequality and quantityin several horticultural crops, mostly cucurbits. The geographical distribution of the virus is not systematical and it depends on climatic conditions. WMV was detected most commonly in temperate climate and subtropic regions. At least 29 species of Aphids, including *Myzus persicae* (Sulzer) and *Aphis craccivora* Koch, transmit the virus in a non-persistent manner (Edwardson and Christie, 1986). It is also transmitted by mechanical inoculation on more than 170 plant species belonging to 27 families (Shukla *et al*., 1994).

The presence of WMV has previously been reported from different parts of Turkey. Nogay and Yorganci (1984) found WMV in 142 of 262 samples tested in Marmara region. Citir *et al*. (1998) detected CMV, ZYMV and WMV in cucurbit crops in the Black Sea region by using symptomatology and biological tests. Sevik and Arli-Sokmen (2003) determined that incidence of WMV on cucurbits was 53.9% (89 out of 165 of samples) in Samsun province. Koklu and Yilmaz (2006) reported that rates of incidence of WMV on watermelon and melon were 34.2% (96 out of 281 samples) and 31.2% (69 out of 221 samples) respectively in Thrace region. There are no reports on the evolution of WMV prevalence since 2006.

Although several studies have been performed on the detection and determination of incidence of WMV on cucurbits in Turkey, it has not been studied extensively at the molecular level. Therefore this study focused on the moleculer characterization of a Turkish isolate of WMV (WMV-Tr) originating from a naturally infected watermelon plant. This paper reports the sequencing and analysis of the part of coat protein gene of a WMV isolate obtained in Adana province in Turkey and examines its relationship to other WMV isolates from different parts of the world.

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Materials and Methods

Virus İsolate and Host Range

WMV-Tr isolate was obtained from a plant with leaf mosaic and deformations in a naturally infected watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) field in Adana province in Turkey. The sample was determined by DAS-ELISA to be devoid of other cucurbit viruses, including PRSV, ZYMV, CMV, squash mosaic virus (SqMV) and cucurbit aphid-borne yellows virus (CABYV). WMV-Tr was maintained on zucchini squash (*Cucurbita pepo* L. cv. 'Eskenderany') and kept in controlled climatic room (25-27ºC).

For host range studies, five plants of each species namely *Cucurbita pepo* L., *Cucumis sativus* L., *Citrullus lanatus* (Thunb.) Matsum. and Nakai**,** *Cucumis melo* L., *Chenopodium amaranticolor* Coste and Reynier, *C. quinoa* Willd., *Nicotiana tabacum* L. cv. Samsun, *N. glutinosa* L. and *N. rustica* L.were inoculated and five uninoculated plants were used as negative control. All plants were kept in the climatic room and investigated daily for symptom expression. The presence of WMV infection in indicator plants was tested by DAS-ELISA using a WMV specific kit (Art. No. 161165, Bioreba, Switzerland). The experiment was repeated twice.

In mechanical inoculation, leaf extract was prepared in cold 0.01 M potassium phosphate buffer, pH 7.0 containing 0.01 M 2-mercaptoethanol (1 g leaf/5ml buffer) and rubbed onto carborundum dusted leaves of indicator plants.

Aphid Transmission Experiments

Aviruliferous adult wingless green peach aphids (*Myzus persicae* (Sulzer)) were used for nonpersistent transmission of WMV-Tr isolate. The aphids were starved for 4 to 5h before 2 min acquisition period. Aphids were then transferred by groups of ten on each of 5 squash plants for a 2h inoculation period. The plants were sprayed with an aphicide and transferred to cages in the climatic room. Symptoms were observed 2 weeks later. The test was repeated three times.

Serological Tests

DAS-ELISA tests were conducted according to the antisera manufacturer's recommendations. ELISA plates (NUNC, Denmark) were coated with WMV specific polyclonal antisera diluted in carbonate buffer. After washing, sample extracts in extraction buffer were added and plates incubated overnight at 4ºC. After washing, alkaline phosphatase conjugated antibody diluted in conjugate buffer was added and the plates were incubated for 4 h at 30ºC. The virus infection was detected by addition of p-nitrophenyl phosphate (1 mg/mL) in substrate buffer after incubating at room temperature for 60-120 min. Absorbance values were measured at 405 nm using a MEDISPEC ESR-200 reader. Samples were considered to be positive when their absorbance values were greater than 3 times that of negative control (Wang and Gonsalves, 1990).

Total RNA Extraction

Total RNA was obtained from fresh leaves of WMV infected squash plant as described by Astruc *et al.* (1996). Plant tissue was triturated in 2 volumes of extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA, 500 mM sodium chloride and 0.1% 2-mercapthoethanol) and centrifuged at low speed in 2 ml eppendorf tube. After addition of 50 µL 20% SDS, the extract was kept at 65ºC for 15 min. Then 250 µL of 6M potassium acetate (pH 6.5) were added and the tubes were transferred on ice for 20 min. After centrifugation, nucleic acids were precipitated with 500 µL ethanol. The pellet was resuspended in 50 µL RNAse free-sterile water.

RT-PCR

RT-PCR reactions were performed in a thermal cycler (Techne TC 4000, Cambridge, UK) using WMV specific primers WMV-5' (5'-GGCTTCTGAGCAAAGATG-3') and WMV-3' (5'-CCCAYCAACTGTYGGAAG-3') (Desbiez *et al*., 2009), which yielded a 408 nucleotide (nt) fragment overlapping the N-terminal part of the coat protein (CP).

The complementary DNA synthesis was done in a PCR tube containing 2 µL of total RNA, 2 µL reverse primer (10 mM) and 20 µL of RNAse free sterile water. This mix was heated at 70ºC for 2 min and cooled for 90 sec. Ten µL of reverse transcriptase buffer (5X), 2 unit of MMLV-RT (Fermentas), 2 unit of RNAse inhibitor (RNAsin, Fermentas), 0.6 µL of dNTPs (25 mM) and 20 µL of sterile water were added and the mix was incubated at 42ºC for 60 min.

PCR was performed using 8 µL of *Taq* DNA polymerase buffer (Fermentas), 2 µL of dNTPs (25 mM), 5 units of *Taq* polymerase (Fermentas), 2 µL of each primer, 33.6 µL of sterile water and 2 µL of the cDNA. PCR reactions were performed by initial denaturation of 2 min at 94ºC followed by 35 cycles of 30 sec at 94ºC, 30 sec at 53ºC and 2 min at 72ºC. Final extension was 10 min at 72ºC. Ten µL of PCR product were analysed using electrophoresis through 1% agarose gel.

Nucleic Acid Sequencing and Sequence Analysis

The amplified fragment of WMV-Tr was purified according to manufacturer's instructions with the Roche High Pure PCR Product Purification Kit. Sequencing was done by using automated DNA sequencer (Aplied Biosystems ABI 310)atIontekResearch and Biotechnology Company (Turkey).

The nt sequence of WMV-Tr was compared with GenBank isolates using BLASTn (Camacho *et al.*, 2008).

Multiple alignment was performed using the program *ClustalW* included in MEGA5 (Tamura *et al.*, 2011). A neighbour-joining tree was built with 500 bootstrap replicates using MEGA5. Isolate names and GenBank accession numbers are shown in Table 1.

Results

Mechanical Inoculation and Aphid Transmission

WMV-Tr isolate was inoculated onto 9 different plant species of 3 different families. It was able to systemically infect all tested cucurbit plants inducing mosaic, mottle, leaf deformation or vein banding of the entire plants. It caused severe mosaic, mottle and leaf deformations in zucchini squash (Fig. 2D), mosaic and vein banding in melon (Fig. 2C), mosaic and leaf deformation in watermelon (Fig. 2B) and mosaic in cucumber (Fig. 2A). In *Chenopodium quinoa* Willd.and *C. amaranticolor* Coste and Reynier, WMV-Tr induced chlorotic local lesions (Fig. 2E and Fig. 2F). Based on symptoms and DAS-ELISA, it was however unable to infect the three *Solanaceae* species tested either locally or systemically (Table 1).

Isolate WMV-Tr was efficiently transmitted by *M. persicae* (Sulzer) with an average transmission rates of 95%, when ten aphids were used on each test plant (*C. pepo* L. cv. 'Eskenderany'). The plants showed mosaic, mottle and leaf deformation symptoms.

Nucleic Acid Sequencing and Sequence Analysis

A fragment of the predicted size, 408 bp, was obtained by RT-PCR with WMV-specific primers. Sequence comparisons showed that the WMV-Tr has 84 to 99% nucleotide sequence identity with WMV isolates from different parts of the world (Table 2). The highest level of nucleotide identity (99%) was observed with an isolate from China (Genbank accession AY464948) whereas the lowest nucleotide sequence identity (<86%) was with isolates from Chile and Tonga (Genbank accessions EU660580 and L22907) (Table 2).

A distance tree was constructed from the sequences of the CP genes of 31 selected WMV isolates together with WMV-Tr isolate. WMV isolates belonged to three main clusters corresponding to groups G1, G2 and G3 already described for this virus (Desbiez *et al.*, 2007) (Fig. 1). Within cluster 3, 4 subclusters (EM1 and to EM4) could be defined as previously observed for WMV (Desbiez *et al.*, 2009). Isolate WMV-Tr clustered with isolates from China, South Korea and France in subcluster EM3 (Fig. 1).

Discussion

In this study, a watermelon mosaic virus (WMV, *Potyvirus*) isolate named WMV-Tr was obtained from a naturally infected watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) plant exhibiting mosaic, mottle and leaf deformation symptoms and characterized biologically and molecularly. It was shown to belong to WMV molecular group G3.

Table 1: Reaction of host plants to sap inoculation by Turkish WMV isolate (WMV-Tr) and ELISA results

Family	Host	Plants Symptoms	ELISA
Cucurbitaceae	*Cucumis sativus L*	M	+
	Citrillus lanatus (Thumb.) (Matsum and Nakai)	M, LD	+
	Cucumis melo L.	M, VB	+
	Cucurbita pepo L.	M, Mo, LD	+
Chenopodiceae	*Chenopodium quinoa Willd.*	CLL	+
	C. amaranticolor (Coste and Reynie)	CLL	+
Solanaceae	*N. tabacum* L.cv. *Samsun*	-	-
	N. glutinosa L.	-	-
	N. rustica L.	-	-

CLL: chlorotic local lesion, LD: leaf deformation, M: mosaic, Mo: mottle, VB: vein banding

Table 2: Genbank accession number and origin of WMV isolates/strains used for phylogenetic comparision of the 348 nt sequence encompassing partial NIb andCP coding regions

Isolate	Origin	Host	Accession number	% identity
WMV-CHN	China	Watermelon	DQ399708	99,1
FBR04-37	France	Zucchini	EU660586	98,0
C06-257	France	Melon	JF273463	97,7
Ch99/69	China	Zucchini	EF127832	97,4
Watermelon	South Korea	Melon	AB369278	97,4
Liaocheng	China	Pumpkin	EF122501	97,4
FMF00-LL1	France	Zucchini	EU660581	92,8
S96-6	Japan	Pumpkin	AB353119	92,5
FMF03-141	France	Zucchini	EU660583	92,2
FMF00-LL2	France	Zucchini	EU660578	90,9
159-08	Serbia	Zucchini	JF325891	90,9
C06-526	France	Melon	JF273462	90,6
IR02-54	Iran	Zucchini	EU660584	90,6
M116	Spain	-	AF551334	90,6
SK02-sq	Slovakia	squash	GQ241710	90,6
C05-270	France	Melon	EU660585	90,5
Lecce	Italy	Watermelon	FJ823122	90,5
C05-337	France	Zucchini	EU660589	90,4
WMV-FR	France	-	NC_006262	90,4
IR03-sq	Iran	Squash	GQ241701	90,4
SK22-cu	Slovakia	Cucumber	GQ241707	90,4
IR11-Cu	Iran	Cucumber	GQ241699	89,8
Turk91	Turkey	-	EU660579	89,8
Chi02-481	Chile	-	EU660582	89,5
New Zealand	New Zealand	-	AY995215	88,3
C06-188	France	Cucumber	EU660587	87,4
Ita00-G	Italy	-	EU660590	86,8
USA	USA	-	D13913	86,8
RobWMV1	USA	*Robinia pseudoacacia*	JN831647	86,0
CHI87-620	Chile	Zucchini	EU660580	86,0
Vanilla	Tonga	Vanilla	L22907	85,7

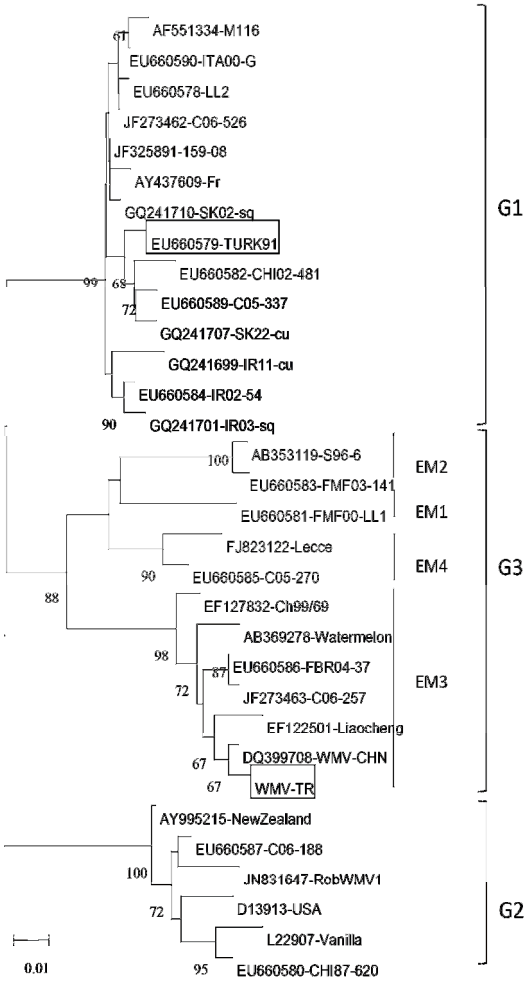

Fig. 1: Neighbour-joining tree constructed with nucleotides sequences from a 348-nt fragment encompassing the C-terminal part of the NIb and N-terminal part of the coat protein of 32 WMV isolates from different parts of the world. Bootstrap values (500 replicates) above 60% are indicated as percentage for each node. Isolates WMV-TR and TURK91 (collected in Turkey in 1991) are indicated in boxes

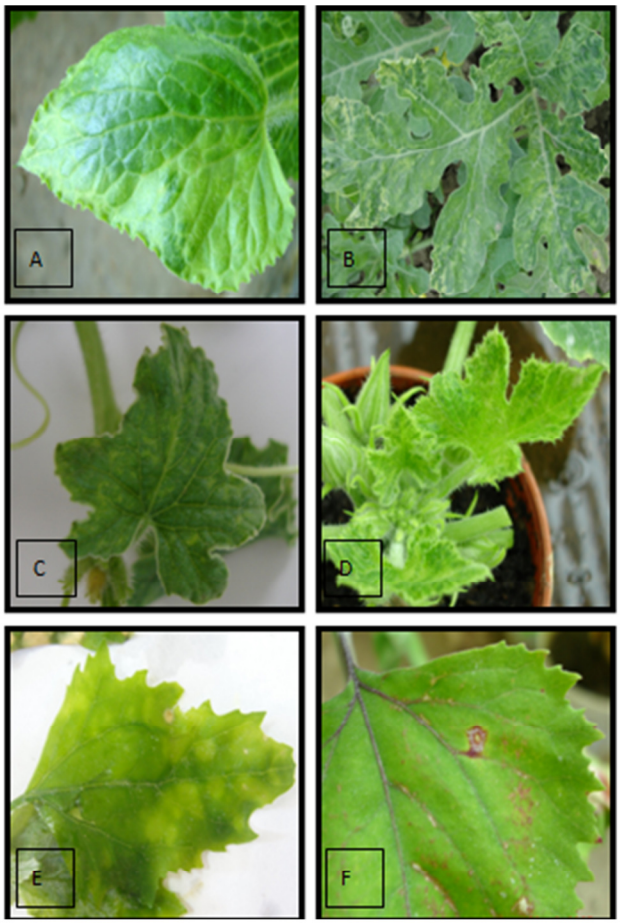

Fig. 2A: Mosaics in cucumber, **B:** Mosaics and leaf deformation in watermelon, **C:** Mosaics and vein banding in melon, **D:** Mosaics, mottle and leaf deformation in zucchini squash, **E:** Chlorotic local lesions in *Chenopodium quinoa Willd*., **F:** Chlorotic local lesions in *Chenopodium amaranticolor Coste* and Reynier

G3 isolates were first described in Asia. In early 2000, G3 isolates, not detected before in this country, were associated with severe symptoms in France. Within a few years, they have almost completely replaced in south-eastern France the G1 isolates present before (Desbiez *et al.*, 2009), probably in relation to a better fitness (Fabre *et al.*, 2010; Lecoq *et al.*, 2011). They have also been reported in the 2000s in other European countries including Italy, Spain and Poland (Borodynko *et al.*, 2009; Lecoq and Desbiez, 2012; Finetti Sialer *et al.*, 2012) as well as in the USA (Vincelli and Seebold, 2009). Within G3 isolates, 4 molecular subgroups (named EM1 to EM4) have been defined. Sequence data placed WMV-Tr in group EM3 that is the least common in south-eastern France (Desbiez *et al.*, 2009), but was the most common type of EM isolates detected in the other European countries (Borodynko *et al.*, 2009). The biological properties of WMV-Tr were in agreement with Purcifull *et al.* (1984). Like the other G3 isolates, WMV-Tr was not systemic on *Chenopodium quinoa* (Lecoq *et al.*, 2011), due to the presence of a "KEKET" motif instead of "KEA" in the N-terminal extremity of its CP (Desbiez *et al.*, 2014). WMV-Tr was efficiently aphid transmitted, in relation to the presence of the "DAG" motif involved in potyvirus transmissibility (Ng and Falk, 2006). This study showed that EM strains of WMV are now present in Turkey.

To our knowledge this is the first detection and molecular characterization of a isolate of "emerging" type of WMV in Turkey. It is difficult to say if this group has been in Turkey for a long time. The only Turkish sequence

obtained from 1991 (accession number EU660579) corresponded to a "Group 1" isolate (Fig. 1). We also don't have any information on the temporal evolution of WMV populations in Turkey. So some further studies on molecular identification and analysis of isolates and strains of WMV and their geographic distributions, collection and evaluation of epidemiological data (overwintering in weeds, transmission by aphid vectors) should be planned and carried out in different major cucurbits growing areas in Turkey.

Conclusion

A WMV isolate named WMV-Tr obtained from symptomatic watermelon in Turkey belonged to molecular group 3 that contains isolates currently emerging in Europe. This suggests that Group 3 is now emerging in Turkey. This is the first detection and molecular characterization of a isolate of 'emerging' type of WMV in Turkey.

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References

Anonymous, 2009. TurkStat, Agricultural Structure, Production, Price, Value. Available at: http: //tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul (Accessed: 15 December 2013)

Astruc, N., J.F. Marcos,G. Macquarie, G.T. Candresse and P. Vicent, 1996. Studies on the diagnosis of hop stunt viroid in fruit trees: Identification of new host and application of a nucleic acid extraction procedure based on non–organic solvents. *Eur. J. Plant Pathol.,* 102: 837–846

Borodynko, N., B. Hasiow–Jaroszewska, N. Rymelska and H. Pospieszny, 2009. Watermelon mosaic virus reported for the first time in Poland. *Plant Pathol*., 58: 783

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer and T.L. Madden, 2008. "BLAST+: architecture and applications." *BMC Bioinformatics* 10: 421

Citir, A., N.D. Kutluk, N. Saglam and H. Ilbagi, 1998. Amasya, Corum, Samsun ve Tokat illerinde hiyar ve kabak kulturlerinde gorulen virus hastaliklarinin simptomatolojik ve biyolojik yontemlerle tanilari. Turkiye VIII. Fitopatoloji Kongresi. Ankara, 331–335 (In Turkish)

Desbiez, C., C. Costa, C. Wipf–Scheibel, M. Girard and H. Lecoq, 2007. Serological and molecular variability of watermelon mosaic virus (genus *Potyvirus*). *Arch. Virol*., 152: 775–781

Desbiez, C., B. Joannon, C. Wipf–Scheibel, C. Chandeysson and H. Lecoq, 2009. Emergence of new strains of watermelon mosaic virus in South–eastern France: Evidence for limited spread but rapid local population shift. *Virus Res.,* 141: 201–208

Desbiez, C., C. Chandeysson and H. Lecoq, 2014. A short motif in the N–terminal part of the coat protein is a host–specific determinant of systemic infectivity for two potyviruses. Molecular Plant Pathology. *Mol. Plant Pathol*., 15: 217–221

Edwardson, J.R., and R.G.Christie,1986. Cucumoviruses. *In: Viruses Infecting Forage Legumes*. pp: 143–215. J.R. Edwardson and R.G. Christie (eds.). Monogram 14, IFAS, University of Florida, Gainesville, Florida, USA

Fabre, F., J.Chadœuf, C.Costa, H. Lecoq and C. Desbiez, 2010. Asymmetrical over–infection as a process of plant virus emergence. *J. Theor. Biol.,* 265: 377–388

Finetti–Sialer, M.M., T. Mascia, F. Cillo, C. Vovlas and D. Gallitelli, 2012. Biological and molecular characterization of a recombinant isolate of *Watermelon mosaic virus* associated with a watermelon necrotic disease in Italy. *Eur. J. Plant Pathol.*, 132: 317–322

Koklu, G. and O.Yilmaz, 2006. Occurrence of cucurbit viruses on field–grown melon and watermelon in the Thrace region of Turkey. *Phytoprotection*, 87: 123–130

Lecoq, H., F. Fabre, B. Joannon, C. Wipf–Scheibel, C. Chandeysson, A. Schoeny and C. Desbiez, 2011. Search for factors involved in the rapid shift in watermelon mosaic virus (WMV) populations in south–eastern France. *Virus Res.,* 159: 115–123

Lecoq, H. and C. Desbiez, 2012. Viruses of cucurbit crops in the mediterranean region: an ever–changing picture. *Adv. Virus Res*., 84: 67–126

Ng, J.C.K. and B.W. Falk, 2006. Virus–vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. *Ann. Rev. Phytopathol*., 44: 183–212

Nogay, A. and U. Yorganci, 1984. Investigations on the identification, seed transmission and host range of viruses infecting the culture plants in the Cucurbitaceae in Marmara region. 1. The identification of viruses infecting cucurbits in Marmara region. *J. Turk. Phytopathol*., 13: 9–28

Provvidenti, R., 1996. Diseases caused by viruses. *In: Compendium of Cucurbit Diseases*. pp: 37–45. T.A. Zitter, D.L. Hopkins and C.E. Thomas (eds.). American Phytopathplogical Society, St. Paul, Minnisota, USA

Purcifull, D., E. Hiebert and J. Edwardson, 1984. *Watermelon Mosaic Virus 2*. CMI/AAB Descriptions of Plant Viruses. No: 293 (no. 63 revised). Kew, Surrey, England

Romay, G., H. Lecoq and C. Desbiez, 2014. Zucchini tigré mosaic virus is a distinct species into the papaya ringspot virus cluster: Molecular and biological insights. *Arch. Virol*., 159: 277–289

Sevik, M.A. and M. Arli–Sokmen, 2003. Viruses infecting cucurbits in Samsun, Turkey. *Plant Dis.,* 87: 341–344

Shukla, D.D., C.W. Ward and A.A. Brunt, 1994. *The potyviridae*. CAB International. Pp: 515.Wallingford, UK

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., 28: 2731–2739

Vincelli, P. and K. Seebold, 2009. Report of a Watermelon mosaic potyvirus Strain in Kentucky Undetected by ELISA. *Plant Health Prog.,* doi: 10.1094/PHP–2009–0313–01–BR

Wang, M. and D. Gonsalves, 1990. ELISA detection of various tomato spotted wilt virus isolates using specific antisera to structural proteins of the virus. *Plant Dis.*,75: 154–158

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